Progress Report for AFOSR on Taiwan – AFOSR Nanoscience Initiative
AOARD project 074082
Project Titles: Preparation and Applications of Active Nanosturcture as Biosensors
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Introduction

Goals: (1) To prepare and characterize ultrastable functionalized mesoporous silicas (MPS) materials with nanochannels (3 – 100 nm). **(2)** To prepare and characterize enzymes and biomimetic complexes confined in MPS. **(3)** To develop these MPS immobilized with enzymes and biomimetic complexes into durable biosensing devices to detect toxins and other health hazardous substances.

The plan was to synthesize active MPS with nanospaces to immobilize proteins (enzymes or biomimicking agents), fragments of DNA or small RNA, and to study their biosensing utility by electrochemical and spectroscopic methods.

During this year, our research has resulted in the following concrete outputs which include three manuscripts submitted to top journals; two of them are already accepted and one is under review. Please notice the first one is an invited review article which gave a rather exhaustive review of the field of Mesoporous Materials for Immobilizing Enzymes.

1. Chia-Hung Lee, Tien-Sung Lin, Chung-Yuan Mou', 2009 **"**Mesoporous Materials for Immobilizing Enzymes" *Nano Today*, accepted
2. Chia-Hung Lee, Han-Chou Lin, Tien-Sung Lin, and Chung-Yuan Mou, 2009 "Hydroxo-Bridged Dinuclear Cupric Complexes Encapsulated in Various Mesoporous Silica to Mimic the Catalytic Activity of Catechol Oxidases: Reactivity and Selectivity Study" *J. Phys Chem C,* submitted
3. Shih-Hsun Cheng, Sergei A. Vinogradov, Chia-Hung Lee, Chung-Shi Yang, Fan-Gang Tseng, Chung-Yuan Mou, and Leu-Wei Lo, 2009 "Mesoporous Silica Nanoparticles Functionalized with Oxygen-Sensing Probe for Cell Photo-Therapy as Potential Cancer Theranostics" *J Mater Chem,* accepted

Summary of the results:

(1) *To prepare and characterize ultrastable functionalized mesoporous silicas (MPS) materials with nanochannels (3 – 100 nm):*

We have made ultrastable hydropphobisized MPS which is employed in research into supercooled water and in part 2 and 3. The hydrophobically functionalized MCM samples were obtained by treating regular, hydrophilic, MCM-41-S, synthesized following a procedure reported elsewhere. The MCM-41-S nanoporous silica matrix has 1D cylindrical pores arranged in 2D hexagonal arrays, with pore diameters characterized by a narrow distribution. The specimen employed for obtaining the hydrophobically modi¯ed MCM samples used in the experiments had a pore diameter of 18 °A(MCM-41-S-18) which is determined by capillary condensation of nitrogen at 77 K using Kelvin equation, the Barrett-Joiner-Halenda(BJH) method. It is known that the BJH method usually underestimates the pore size (by ¼ 20 %). So the pore size given here is a thermodynamic estimate. By treating the hydrophilic MCM-41-S-18 samples with Chloro-

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14. ABSTRACT
This project synthesized active mesoporous silicas with nanospaces to immobilize proteins or fragments of DNA to determine their biosensing utility via electrochemical and spectroscopic methods.

15. SUBJECT TERMS
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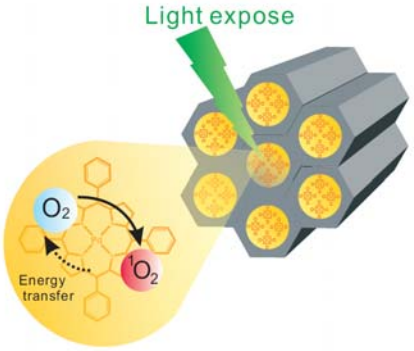
Trimethyl Silane (CTS), the silanol –OH groups which cover the walls of the pore can be changed to methyl groups (-O-Si(CH3)3). By varying the CTS/MCM ratio di®erent levels of reaction can be achieved and therefore various degrees of hydrophobicity. In the present case a 1:1 ratio of MCM to CTS was used in the synthesis gel. In the following of the paper, we will use the name MCM-41-SM1-18 for the hydrophobically modified sample thus obtained. The amount of CTS attached to the surface of the pores has been measured by elemental analysis yielding 1.62£10¡6 mol of CTS per square meter, or 0.98 CTS molecules per square nanometer. The saturation monolayer coverage of CTS is 2.4 molecules per square nanometer. Therefore, the sample investigated in the present paper has 42 % of the pore surface covered by hydrophobic methyl groups. It should be noted that after the treatment with CTS the size of the pores is reduced to 15 ^{o}A because of the presence of the trimethyl silyle groups. We note here that although the absolute value of pore diameter is somewhat uncertain, this sample of hydrophobic nature possess the same pore diameter of our hydrohillic MCM-41-S-15 preiously used for confining water. The samples were hydrated by exposing them to water vapor in a closed container and a hydration level of about 0.25 mass fraction (mass of water/sample total mass) was achieved. The materials is very stable to water as tested at boiling temperature for one week.

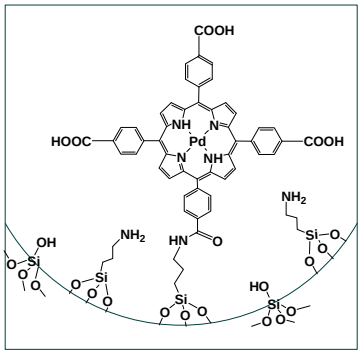
(2) *To prepare and characterize enzymes and biomimetic complexes confined in MPS.*

Although we proposed to study biomic of the enzyme tyrosinase, we finally decide to work on catechol oxidase. Both are di-copper complex and both performed the oxidation of catechol. So the final output is close to the original goal. We report the synthesis and characterization of two hydroxo-bridged dinuclear cupric complexes, HPC $(((phen)_2Cu\text{-}OH\text{-}Cu(phen)_2)^{3+}$, phen = 1,10-phenanthroline) and HBC $(((bpy)_2Cu\text{-}OH\text{-}Cu(bpy)_2)^{3+}$, bpy = 2,2'-bipyridine), encapsulated in porous materials for the oxidation of 3,5-di-*tert*-butylcatechol (DTBC) to the corresponding quinone, 3,5-di-tert-butylquinone (DTBQ) to mimic catechol oxidases (COs). The separations of two Cu(II) centers are 2.9, 3.51 and 3.65 Å for CO, HPC and HBC, respectively. The stability of dinuclear cupric complexes, turnover number (TON) and selectivity of DTBQ were examined in NaY zeolite (pore size 0.74 nm), and mesoporous silica (MPS): MCM-41 (2.4 nm), MCM-48 (2.5 nm), and MAS-9 (9.0 nm) solids. The studies showed that the MCM-41 and MCM-48 provided a better stability against the irreversible dissociation of dinuclear cupric complexes for their matching size, while NaY has too small and MAS-9 has too large pore size to stabilize these dinuclear copper complexes. The EPR studies showed that HBC immobilized in MPS solids yielded more mononuclear cupric complexes than HPC samples which may come from the low stability of HBC undergoing the dissociation of OH bridge via the Lewis acid (aluminum sites in the solid support) catalytic activities under the ion-exchanging process. The catalytic pathways for the production of DTBQ and byproducts are proposed based on spectroscopic characterizations and activity measurements. The main byproduct observed in NaY supports was formed from a DTBC-mononuclear copper intermediate and followed the pathway of electron transfer, oxygen insertion, ring opening, and oxidation reaction. Furthermore, the rigid and bulky structure of HPC molecule (planar phen ligands) has more confinement effect in MCM-41 and MCM-48 solids than the flexible HBC molecule (nonplanar bpy) which can prevent an excessive separation of the dinuclear cupric centers in the deoxy state and yield a higher stability and selectivity. The smaller separation of two Cu(II) ions in HPC may also be responsible for the observed higher oxidation selectivity. However, the bulky structure of four phen ligands in HPC molecules exhibits greater steric hindrance and decreases the contact of the substrate and yields a lower TON. The nanochannels of aluminum substituted MPS provide the needed confined spaces and surface charge, and maintain the separation of the dinuclear cupric centers after removing the hydroxo-bridge in the catalytic cycle.

(3) *To develop these MPS immobilized with enzymes and biomimetic complexes into durable biosensing devices to detect toxins and other health hazardous substances.*

We have developed oxygen sensor based on functionalized MPS which can also be used in phtodynamic therapy. Mesoporous silica nanoparticles (MSNs) functionalized with Pd porphyrin for cancer cell photo-therapy is reported. This MSNs platform extends the Pd-porphyrin from its renowned function as a phosphorescence probe for oxygen sensing/imaging (diagnostics) to a novel nano-photosensitizer for cell photo-therapy (therapeutics). The efficacy of photo-therapy against MDA-MB-231 breast cancer cells is also demonstrated in current study. The Pd-porphyrin functionalized MSNs presents a promising platform for cancer theranostics.





In summary, much of the original goals in the proposal have been achieved and several papers will be published in high profile journal. Complete papers can be supplied upon request.